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ORIGINAL ARTICLE

Tumor Necrosis Factor-A, Interleukin-6, C-Reactive Protein Levels and Insulin Resistance Associated with Type 2 Diabetes in Abdominal Obesity Women

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Abstract We aim to investigate the association between elevated tumor necrosis factor-α (TNF-α), interleukin-6 (IL-6) and high sensitivity-C-reactive protein (hs-CRP) with type 2 diabetes mellitus (T2DM) in abdominal obesity (AO) women subjects. A total of 428 AO subjects (age 48.4 ± 10.2 years), and 107 non-AO women subjects (age 48.8 ± 11.8 years) were enrolled for the all biochemistry testing, inflammatory cytokines, fasting insulin and Homeostasis Model Assessment of insulin resistance (HOMA-IR). Body mass index, waist circumference (WC), blood pressure (BP), plasma glucose (Glu), triglyceride (TG), insulin, HOMA-IR and inflammatory cytokines were significantly higher and lower total antioxidant capacity, HDL-C in AO subjects ($p < 0.05$). WC was significantly correlated with BP, Glu, TG, LDL-C, insulin, HOMA-IR, TNF-α, IL-6 and negative correlation with HDL-C in AO subjects. Elevation of TNF-α, IL-6, hs-CRP and insulin resistance were significantly associated with T2DM in AO subjects, after adjusting with insulin resistance, increased oxidative stress, elevated TG and reduced HDL-C by using multiple logistic regression analysis. In conclusions, elevation of inflammatory cytokines, oxidative stress and insulin resistance were associated with T2DM in AO women subjects. These inflammatory cytokines are positively associated with T2DM and may have a causal relation with an increased oxidative stress and insulin resistance in these AO women subjects.

Keywords Abdominal obesity · Insulin resistance · Tumor necrosis factor- α · Interleukin-6 · hs-CRP · Type 2 diabetes mellitus

Introduction

Abdominal obesity (AO) may indicate an individual has visceral adipose tissue around the stomach and abdomen. Also known as central obesity, may indicate an individual's visceral adipose tissue is a calorie surplus resulting from excess energy intake and/or reduced energy expenditure, increasing the risk of cardiovascular disease (CVD) and type 2 diabetes mellitus (T2DM) [1]. Visceral adipocytes play important endocrine roles in the process of inflammation and worsening insulin sensitivity [2]. Thus, concept of obesity is as an inflammatory state and these inflammatory cytokines of the process have been identified. These inflammatory cytokines may serve as reasonable screening tools. In response to excess lipid stores, visceral adipocytes secrete increasing amounts of inflammatory cytokines such as tumor necrosis factor-α (TNF-α), interleukin-6 (IL-6) and chemokines such as monocyte chemoattractant protein-1 [3]. These cytokines in turn promote the migration of macrophages to the adipose tissue further increasing cytokine release. These proinflammatory conditions conferred by excess visceral adipose tissue combine to produce a tonic degree of systemic inflammation. These processes appear to play a central role in the development of insulin resistance and future diseases, as supported by basic

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science and clinical information for screening and risk assessment.

The most commonly used marker to assess systemic inflammation is C-reactive protein (CRP). CRP is produced by the liver, peripheral leukocytes, and even the adipose tissue in response to multiple cues, particularly increases in IL-6 and other systemic inflammatory cytokines [2]. Human data linking inflammation to insulin resistance that demonstrated inter-relations between systemic markers of inflammation (CRP) and the presence of insulin resistance in adults [4, 5] and adolescents [6, 7]. Obesity results in insulin resistance [8–10]. Although certain proportions of cardiovascular disorders are attributed to the secondary complications of obesity (e.g., hypertension, atherosclerosis, T2DM and aging), a direct deleterious effect of obesity on the cardiovascular system is now clearly evident. Inflammatory mechanisms play a key role in the pathogenesis of diabetes mellitus. Individuals who progress to T2DM display features of low-grade inflammation before the onset of diabetes. This low-grade inflammation has been suggested as being involved in the pathogenetic processes that cause T2DM [1]. Several humoral markers of inflammation are elevated in humans with T2DM [3]. All of these findings prompted us to hypothesize that elevated TNF-α, IL-6, CRP and insulin resistance are associated with T2DM in AO subjects.

Materials and Methods

Study Population

Subjects

This cross-sectional study was performed by using data from a Health Survey for Protection of hypertension and T2DM (age ≥ 40 years) from three districts of Khampangpetch and Phitsanulok Province (February 2011–January 2013). 428 of AO participants (age 48.4 ± 10.2 years) and 107 non-abdominal obesity (nAO) (age 48.8 ± 11.8 years) participated in the present study. Among these subjects, 20 women had reached menopause during the survey period. The duration of menopause was variable, going from 2 months to 25 years. All women were subjected to a medical examination. 160 women used antihypertensive medication and ten women were just diagnosed. 77 women used antihyperglycemic medication, and they were kept in the study. We excluded the 126 subjects with known end stage renal failure, cancer, infection and any life threatening diseases from the study. All eligible participants were apparently healthy with no clinical signs of associated pathologies, organ damage, no history of coronary, cerebrovascular atherosclerotic disease, recurrent or a past history of psychiatric illness, significant medical disorder, drug, cigarette or alcohol abuse. All participants gave written informed consent and they all agreed to participate and to provide blood sample for their health check. The Ethics Committee of Naresuan University approved the study protocol.

Anthropometric and Blood Pressure Measurement

Subjects' height, weight, and blood pressure (BP) were measured and body mass index (BMI) was calculated. Waist circumference (WC) was measured at the midpoint between the both of rib cage and the top of lateral border of iliac crest during minimal respiration. AO defined as WC ≥ 80 cm or 31.5 inches (female) [11]. BP was measured after the participants were seated and rested for 5 min as the mean value of at least two measurements of these participants on the same day with a Terumo digital BP monitor (ES-P110). Hypertension was defined as an average BP ≥ 140/90 mmHg or if the participant was taking antihypertensive medications or had been diagnosed with HT [12, 13]. Diabetes was defined as a fasting glucose concentration of ≥ 126 mg/dl, a non-fasting glucose concentration of ≥ 200 mg/dl, a self-reported physician diagnosis or medication use.

Blood Sample Collection and Biochemical Determination

Venous blood samples were collected without stasis after a 12 h fast and a 30 min rest in a supine position. Blood specimens were processed and assayed on the central laboratory of Department of Medical Technology, Faculty of Allied Health Sciences on the same day. Fasting plasma glucose (Glu), total cholesterol (TC), triglycerides (TG), high density lipoprotein cholesterol (HDL-C) and low density lipoprotein cholesterol (LDL-C) was calculated by Friedewald's equation, which is valid for TG values less than or equal to 400 mg/dl.

Insulin Assay

Fasting insulin levels were measured based on micro-particle enzyme immunoassay technology using Abbott reagents with Axsym system (Abbott laboratories, Illinois, USA). All participants underwent evaluation of Homeostasis model assessment (HOMA)-formula for insulin resistance index (HOMA-IR), HOMA %B [as beta cell function (insulin activity)], and Quantitative Insulin Sensitivity Check Index (QUICKI; as insulin sensitivity) [13–15]. HOMA-IR was defined using the following formula: fasting glucose

(mmol/l) × fasting insulin (μU/ml)/22.5. HOMA %B as formula: [20 × insulin (μU/ml)]/[glucose (mmol/l) − 3.5]. QUICKI as formula: 1/[LOG (insulin (μU/ml)] + LOG [glucose (mmol/l)].

Malondialdehyde (MDA) Assay

The method is based on the formation of red (pink) chromophore following the reaction of thiobarbituric acid (TBA) with MDA and the other breakdown products of peroxidized lipids called thiobarbituric acid reactive substance. One molecule of MDA reacts with two molecules of TBA to yield a pink pigment with maximum absorption at 532 nm. This was measured by spectrophotometry using 1,1,3,3-tetraethoxypropane as standard as described previously [16]. The final results were expressed as μmol of MDA formed per liters of serum. Intra-assay and inter-assay imprecision were 3.24 and 5.78 %, respectively. The normal range of MDA was < 3.5 μmol/L.

Total Antioxidant (TAC) Status

The method is based on formation of the $ABTS^{\bullet+}$ cation [2, 2′-azinobis (3-ethylbenzothiazoline-6-sulfonic acid)] and its scavenging by antioxidant sample constituents (serum) measured by spectrophotometry at 600 nm decay of green/blue color absorption is inversely associated with antioxidant sample content and the control antioxidant is Trolox, a hydrophilic vitamin E analog [17].

Systemic Inflammation Assays

The concentrations of IL-6, TNF-α determined using the ELISA assay kits purchased from Invitrogen (Carlsbad, CA). Standard curves were constructed for determination of each analyte concentration according to the manufacturers' instructions. In accordance with standard practice, a protocol provided by Invitrogen for custom assays was used with no modifications. High sensitivity CRP (hs-CRP) concentrations were determined by using latex-enhanced immunoneplelometric assay on the Hitachi 912 auto-analyzer (Roche Diagnostic, Switzerland) that has been standardized against the World Health Organization reference. The normal range of hs-CRP was < 3.0 mg/l.

Statistical Analysis

Categorical data are presented as percentages. Mean and standard deviation were used for normally distributed data while median and interquartile range was used for non-normally distributed data, as determined by using Shapiro–Wilk test. Comparisons between groups were performed by using a Student's *t* test for normally distributed data and Mann–Whitney Test for non-normally distributed data. The correlation between all variables was analyzed by Spearman's correlation. Odds ratios from logistic regression analyses were used to estimate the elevated TNF-α, IL-6, hs-CRP, insulin resistance, adjusting with the covariate associated with T2DM in AO women subjects. The results of all analyses were evaluated for statistical significance using p-value < 0.05 and the 95 % confidence intervals (CI). All analysis was performed using the SPSS computer program version 13.0 (SPSS, Chicago, IL).

Results

A total of 428 AO women [median aged 48.0 (42.0–56.0) years] and 107 nAO women (aged 41.5–58.3 years) participated as control in this study. The characteristics of the study population are shown in Table 1. AO women were significantly higher in BP, BMI, WC, Glu, TG, LDL-C, TNF-α, IL-6, hs-CRP, insulin, HOMA-IR, HOMA %B, and lower in HDL-C concentration, TAC and QUICKI ($p < 0.05$). Bivariate correlation between parameters in AO subjects as: WC showed the positive correlation with SystBP ($r = 0.259$, $p < 0.001$), DiastBP ($r = 0.216$, $p < 0.001$), Glu ($r = 0.134$, $p = 0.003$), TC ($r = 0.111$, $p = 0.013$),TG ($r = 0.177$, $p < 0.001$), LDL-C ($r = 0.134$, $p = 0.003$), insulin ($r = 0.388$, $p < 0.001$), HOMA-IR ($r = 0.386$, $p < 0.001$), TNF-α ($r = 0.393$, $p < 0.001$), IL-6 ($r = 0.396$, $p < 0.001$), and negative correlation with HDL-C ($r = -0.102$, $p = 0.023$), TAC ($r = -0.103$, $p = 0.021$). While the other significant bivariate correlation were show in Table 2. Multiple logistic regression analysis were used to test for association between elevated TNF-α, IL-6, hs-CRP, insulin resistance with T2DM in AO subjects. Our study demonstrated that risk factors that significantly affect T2DM are elevated TNF-α, IL-6, hs-CRP and insulin resistance in AO subjects as shown in Table 3 (a–c).

Discussion

Our study demonstrated that AO women was elevated in BP, BMI and WC, Glu, TG and LDL-C, insulin, insulin resistance, increased β-cell function (HOMA %B), TNF-α, IL-6, hs-CRP, and decreased in HDL-C, insulin sensitivity (QUICKI) and TAC. AO is the one major risk factor of metabolic syndrome (MetS). MetS is a cluster of cardiovascular risk factors characterized by visceral obesity, dyslipidemia (low levels of HDL-C and elevated TG levels), hypertension, and glucose intolerance (insulin resistance), and also characterized by insulin resistance and low-grade inflammation with abnormal adipokine

Table 1 Comparison of general characteristics of the abdominal obesity with non-abdominal obesity women

Variables	Abdominal obese (n = 428)	Non-abdominal obese (n = 107)	*P* value
Age (years)	48.4 ± 10.2	48.8 ± 11.8	0.720
Systolic BP (mmHg)	127.0 (115.0–139.0)*	116.0 (104.0–132.0)*	<0.001
Diatolic BP (mmHg)	79.0 (72.0–85.0)	74.0 (66.0–82.0)	<0.001
BMI (kg/m^2)	27.1 (24.7–29.6)	21.7 (19.9–23.4)	<0.001
WC (cm)	91.0 (84.0–94.0)	76.0 (71.0–77.0)	<0.001
Glu (mmol/l)	5.28 (4.91–5.72)	5.17 (4.68–5.45)	0.011
TC (mmol/l)	5.52 (4.82–6.41)	5.21 (4.72–6.04)	0.089
TG (mmol/l)	1.77 (1.18–2.54)	1.16 (0.86–1.84)	<0.001
HDL-C (mmol/l)	1.46 (1.26–1.72)	1.62 (1.39–1.94)	0.001
LDL-C (mmol/l)	3.80 (2.94–4.84)	3.51 (2.59–4.43)	0.013
MDA (μmol/l)	4.22 (3.33–5.27)	4.18 (3.32–5.27)	0.432
TAC (μmolTrolox Equiv/L)	565.1 (393.8–856.2	650.7 (537.1–903.2)	0.002
TNF-α (pg/ml)	3.98 (2.43–5.01)	2.78 (2.22–4.11)	<0.001
IL-6 (pg/ml)	2.78 (1.24–3.54)	1.65 (1.13–2.99)	<0.001
hs-CRP (mg/l)	2.41 (1.05–4.54)	1.29 (0.68–2.28)	<0.001
Insulin (pmol/ml)	7.7 (5.1–11.9)	4.6 (3.4–7.5)	<0.001
HOMA-IR	1.87 (1.12–2.85)	0.90 (0.69–1.55)	<0.001
HOMA %β	87.3 (57.8–140.2)	63.3 (39.6–96.6)	<0.001
QUICKI	0.347 (0.325–0.373)	0.377 (0.357–0.404)	<0.001
Hypertension	148 (34.6 %)	22 (20.6 %)	<0.001
Type 2 diabetes mellitus	72 (16.8 %)	5 (4.7 %)	<0.001

* Data are median (interquartile range) of variables with a skewed distribution

production [18]. Adipose tissue produces numerous adipokines such as adiponectin, leptin and cytokines such as TNF-α and IL-6. High sensitivity-CRP is a sensitive general marker of low-grade tissue inflammation that is not only associated with features of insulin resistance, but has also independently predicted development of hypertension, MetS, T2DM and CVD [19, 20]. IL-6 has been suggested to promote hs-CRP production by the liver as a component of the sub-clinical inflammation related to obesity [21].

Our study also demonstrated decreased TAC but not significantly difference in MDA (lipid peroxidation), it may result from increased in oxidative stress in AO subjects. More important, increasing ROS may result in ultimately resulting in cytotoxic end products [22, 23]. The development of vascular oxidative stress and activation of systemic inflammatory pathways play a major role in the pathogenesis of CVD. Total antioxidant status is a measure of the net effect of the interactions between ROS and antioxidants in circulation, assessing the ability of the antioxidants to inhibit the formation of a specific radial [24]. This method allows for assessment of the status of the antioxidants' ability to work in synergism to reduce the potential ROS damage. Although this method does not give information on specific antioxidants, it provides an overall measure of antioxidant status.

Many studies have been suggested that T2DM is a disease of the innate immune system responsible for the ongoing cytokine mediated acute phase response and low-grade chronic inflammation, which may be involved in the progression of atherosclerosis [25]. Therefore, signs of the activation of innate immune system are occur before the onset of T2DM. Epidemiological evidence and the present study demonstrated that elevated inflammatory markers such as TNF-α, IL-6 and hs-CRP predict the development of T2DM and glucose disorders [26]. TNF-α is a multifunctional regulatory cytokine that changes the expression of several adipocyte-secreted factors including adiponectin and IL-6. In addition, it has been considered an important link to obesity and insulin resistance [27, 28], which were also elevated in these AO women subjects.

TNF is known to impair insulin receptor signaling [29]. TNF-α also inhibits lipoprotein lipase (LPL) and stimulates lipolysis in adipocytes [30], and the resulting increase in circulating nonesterified fatty acids would be expected to contribute to insulin resistance [31]. Another adipocyte secretory product that may be involved in insulin resistance is IL-6, which is a cytokine secreted by many cells, including adipocytes and adipose stromal cells [32, 33]. Like TNF-α and IL-6 inhibit the expression of LPL, but, unlike TNF-α, IL-6 does not stimulate lipolysis [34, 35]. IL-6 secretion is increased in the adipocytes of obese subjects [36] and may be important either as a circulating hormone or as a local regulator of insulin action. TNF-α expression by adipose tissue, several lines of evidence have

Table 2 Bivariate correlation between parameters in abdominal obesity subjects using Spearman rank correlation

Correlation between parameters		Correlation coefficient		Correlation between parameters		Correlation coefficient	
		r	*p* value			r	*p* value
Age	SystBP	0.278	<0.001	Glu	TG	0.188	<0.001
	Glu	0.257	<0.001		HDL-C	−0.115	0.010
	UA	0.117	0.016		UA	0.219	<0.001
	TG	0.176	0.018		Insulin	0.116	0.009
	LDL-C	0.147	0.002		IL-6	0.142	0.003
WC	SystBP	0.259	<0.001		TNF-α	0.119	0.014
	DiastBP	0.216	<0.001	TC	TAC	0.089	0.047
	Glu	0.134	0.003		HDL-C	0.264	<0.001
	TC	0.111	0.013	TG	MDA	0.165	<0.001
	TG	0.177	<0.001		HDL-C	−0.521	<0.001
	HDL-C	−0.102	0.023		Insulin	0.110	0.014
	LDL-C	0.134	0.003		HOMA-IR	0.147	<0.001
	TAC	−0.103	0.021		TNF-α	0.187	<0.001
	Insulin	0.388	<0.001		IL-6	0.181	<0.001
	HOMA-IR	0.386	<0.001		hs-CRP	0.140	0.002
	TNF-α	0.393	<0.001	LDL-C	MDA	0.172	<0.001
	IL-6	0.396	<0.001		Insulin	0.169	<0.001
HDL-C	Insulin	−0.170	<0.001		HOMA-IR	0.176	<0.001
	HOMA-IR	−0.187	<0.001		TNF-α	0.099	0.026
	TNF-α	−0.159	0.001		IL-6	0.097	0.030
	IL-6	−0.150	0.002	IL-6	Insulin	0.561	<0.001
	hs-CRP	−0.101	0.025		HOMA-IR	0.567	<0.001
	TAC	0.109	0.024		TNF-α	0.957	<0.001
	UA	−0.188	<0.001		hs-CRP	0.698	<0.001
MDA	TNF-α	0.140	0.004	TNF-α	Insulin	0.546	<0.001
	IL-6	0.135	0.005		HOMA-IR	0.553	<0.001
TAC	TNF-α	−0.119	0.014		hs-CRP	0.707	<0.001
	IL-6	−0.101	0.035	Insulin	HOMA-IR	0.943	<0.001
	hs-CRP	−0.122	0.007		hs-CRP	0.179	<0.001
				hs-CRP	HOMA-IR	0.182	<0.001

suggested that TNF-α overproduction by adipose tissue may be involved in the pathogenesis of the insulin resistance of obesity. Some authors have found increased serum TNF-α and IL-6 concentrations in T2DM and impaired glucose tolerance (IGT) test subjects [37, 38] as in our present study, but Choi et al. [39] have not found any association of TNF-α and IL-6 with IGT.

Systemic inflammation is characterized by the production of proinflammatory cytokines, TNF-α, IL-6 and CRP. We also demonstrated that AO subjects elevated in TNF-α, IL-6 and hs-CRP levels. There is evidence to implicate TNF-α in the pathogenesis of the link between obesity and T2DM [40]. The most consistent relationship between cytokine expression and obesity-related insulin resistance involved increased TNF-α secretion from adipose tissue and increased IL-6 levels. Elevated TNF-α and IL-6 expression was found in subjects who were only moderately obese. Thus both TNF-α and IL-6 were associated with both obesity and insulin resistance.

In Conclusion

Many of the processes leading to CVD and T2DM are present already in AO subjects, although the likelihood of developing disease appears higher for some AO subjects than others. Screening adults for MetS-related risk for CVD and T2DM may help in identifying individuals at particularly great need for lifestyle modification. Increased TNF-α, IL-6, hs-CRP and insulin resistance associated with T2DM in AO subjects. Systemic inflammatory cytokine are used as the screening tool in adults of intermediate risk for

Table 3 Impact of the association of AO with elevated TNF-α after adjusting for their covariates in a representative sample of the study subjects

Variables	Type 2 diabetes mellitus		
	OR	95 % CI	*p value*
(A)			
Elevated TNF-α	1.79	1.09–2.97	0.022
Insulin resistance	3.15	1.88–5.27	<0.001
Increased MDA	1.61	1.10–2.36	0.013
Increased TG	1.08	0.72–1.62	0.701
Reduced HDL	1.22	0.79–1.90	0.374
Abdominal obesity	1.75	1.03–2.97	0.040
Age	0.99	0.97–1.01	0.410
(B)			
Elevated IL6	1.74	1.04–2.91	0.033
Insulin resistance	3.12	1.91–5.10	<0.001
Increased MDA	1.84	1.26–2.68	0.002
Increased TG	1.13	0.75–1.69	0.557
Reduced HDL	1.32	0.85–2.05	0.224
Abdominal obesity	1.96	1.16–3.29	0.011
Age	0.99	0.97–1.01	0.207
(C)			
Elevated hs-CRP	1.79	1.08–2.95	0.023
Insulin resistance	2.07	1.28–3.34	0.003
Increased MDA	1.58	1.08–2.29	0.017
Increased TG	1.17	0.78–1.74	0.443
Reduced HDL	1.23	0.79–1.91	0.351
Abdominal obesity	2.12	1.25–3.60	0.005
Age	0.99	0.97–1.00	0.140

Model after adjusted for insulin resistance, reduced HDL, reduced TAC and age

CVD and T2DM to trigger more immediate intervention. However, data regarding TNF-α, IL-6 and hs-CRP, systemic inflammatory cytokines are similarly correlated to insulin resistance and T2DM in AO subjects. Such tools for identifying future risk are needed to better motivate patients and their physicians toward lifestyle changes- and toward effective decreases in risk.

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